Customer No. 36441

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
MARY E. BAK
HOWSON AND HOWSON
SPRING HOUSE CORPORATE CENTER
P.O. BOX 457
SPRING HOUSE, PA 19477

RECEIVED
AUG 24 2004
PCT

ENTERED
DUE 9/16/04

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing *(day/month/year)*	16 AUG 2004
Applicant's or agent's file reference PST-0055WO	REPLY DUE within 1 months/days from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US03/16214	16 June 2003 (16.06.2003)	17 June 2002 (17.06.2002)

International Patent Classification (IPC) or both national classification and IPC

IPC(7): C12Q 1/68; A01N 43/04; C07H 21/04; A61K 31/07 and US Cl.: 435/6, 91.1, 325, 375; 514/44, 536/24.5, 23.1, 24.3, 24.33

Applicant

ISIS PHARMACEUTICALS INC.

1. This written opinion is the first (first, etc,) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2 (a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension. See rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 17 October 2004 (17.10.2004)

Name and mailing address of the IPEA/US
Mail Stop PCT, Attn: IPEA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 872-9306

Authorized officer
Terra C. Gibbs *T. Roberts for*
Telephone No. (571) 272-1600

WRITTEN OPINION

International application No.
PCT/US03/16214

I. Basis of the opinion

1. With regard to the **elements** of the international application:*

☒ the international application as originally filed

☒ the description:
pages 1-127, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the claims:
pages 128-136, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☐ the drawings:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

☒ the sequence listing part of the description:
pages 1-88, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____.

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

☒ contained in the international application in printed form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages NONE _____

☐ the claims, Nos. NONE _____

☐ the drawings, sheets/~~fig~~ NONE _____

5. ☐ This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed."*

WRITTEN OPINION

International application No.
PCT/US03/16214

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims	28-48, and 50-65	YES
	Claims	1-27 and 49	NO
Inventive Step (IS)	Claims	28-48, and 50-65	YES
	Claims	1-27 and 49	NO
Industrial Applicability (IA)	Claims	1-65	YES
	Claims	NONE	NO

2. CITATIONS AND EXPLANATIONS
Please See Continuation Sheet

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

TIME LIMIT:
The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

V. 2. Citations and Explanations:

Claims 1-65 meet industrial applicability as defined by PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

Claims 28-48 and 50-65 meets the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a method of treating a human having a disease or condition associated with Extracellular-signal-regulated kinase-6 using antisense compounds encoding Extracellular-signal-regulated kinase-6.

Claims 1-27 and 49 lack novelty under PCT Article 33(2) as being anticipated by anticipated by Mercola, D. [U.S. 2002/0107218 A1].

Mercola, D. discloses compositions containing an antisense SAPK3 (also known as extracellular-signal-regulated kinase-6) nucleic acid molecule and a carrier that is acceptable for administration. Mercola, D. further disclose and claim an antisense nucleic molecule is a polymer of above twelve to fifty nucleotides, generally about fifteen to thirty-five nucleotides and usually about twenty to twenty-five nucleotides (see page 4 [0031], claims 1-4 and 11-14). Mercola, D. further discloses antisense molecules containing internucleoside linkages, phosphorothioate bonds and chimeric backbones are useful in the invention (see page 5 [0037] [0039] and [0040]). Mercola, D. further discloses a chemically synthesized antisense nucleic acid molecule can be introduced into a cell (see page 5 [0041]). Mercola, D. further disclose and claim a "SAPK inhibitory agent" can be an antisense SAPK... a SAPK inhibitory agent can be formulated as a pharmaceutical composition, which contains the agent and a pharmaceutically acceptable carrier (see page 7 [0054] and claims 21-24).

Claims 1-27 and 49 lack an inventive step under PCT Article 33(3) as being obvious Mercola, D. [U.S. 2002/0107218 A1], Dinev et al. (EMBO Reports, 2001 Vol. 2:829-834) and Lechner et al. [U.S. Patent No. 6,030,822] in further view of Baracchini et al. [U.S. Patent No. 5,801,154] and Fritz et al. (Journal of Colloid and Interface Science, 1997 Vol. 195:272-288).

Mercola, D. discloses compositions containing an antisense SAPK3 (also known as extracellular-signal-regulated kinase-6) nucleic acid molecule and a carrier that is acceptable for administration. Mercola, D. further disclose and claim an antisense nucleic molecule is a polymer of above twelve to fifty nucleotides, generally about fifteen to thirty-five nucleotides and usually about twenty to twenty-five nucleotides (see page 4 [0031], claims 1-4 and 11-14). Mercola, D. further discloses antisense molecules containing internucleoside linkages, phosphorothioate bonds and chimeric backbones are useful in the invention (see page 5 [0037] [0039] and

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

[0040]). Mercola, D. further discloses a chemically synthesized antisense nucleic acid molecule can be introduced into a cell (see page 5 [0041]). Mercola, D. further disclose and claim a "SAPK inhibitory agent" can be an antisense SAPK... a SAPK inhibitory agent can be formulated as a pharmaceutical composition, which contains the agent and a pharmaceutically acceptable carrier (see page 7 [0054] and claims 21-24).

Dinev et al. teach ERK5 (also known as extracellular-signal-regulated kinase-6) is required for differentiation of muscle cells (see Abstract). Dinev et al. further teach ERK5 endogenous protein is inhibited and myogenic differentiation is blocked when ERK5 expression is inhibited by an undisclosed ERK5 antisense nucleic acid (see Figures 4A and 4B, respectively).

Lechner et al. disclose oligoribonucleotides, including antisense RNA and DNA molecules and ribozymes that function to inhibit translation of one or more components of ERK5 (see column 23, lines 19-66). Lechner et al. generally disclose antisense ERK5 nucleic acid constructs. The ERK5 nucleic acid disclosure of Lechner et al. is almost 100% homologous to SEQ ID NO: 4 of the instant invention (see Lechner et al. SEQ ID NO:2). Lechner et al. further disclose various modifications to the DNA molecule may be introduced as a means of increasing intracellular stability and half-life... possible modifications include the use of phosphorothioate or 2'-O-methyl linkages (see column 24, lines 1-9). Lechner et al. further disclose the use of pharmaceutically acceptable carriers to formulate the compounds disclosed for the practice of dosages suitable for systemic administration (see column 27, lines 11-24).

Mercola, D., Dinev et al. and Lechner et al. do not teach wherein the sugar moiety is a 2'-O-methoxyethyl sugar moiety; wherein the modified nucleobase is a 5-methylcytosine; and a pharmaceutically acceptable carrier or diluent, further comprising a colloidal dispersion system.

Baracchini et al. teach modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases. Baracchini et al. further teach antisense oligonucleotides with at least one modified sugar moiety and a modified 2'-O-methoxyethyl sugar moieties (see Table I)... with modified nucleobases, such as 5-methylcytosine (see column 7, lines 15-25).

Fritz et al. teach a composition comprising an antisense oligonucleotide and a pharmaceutically acceptable carrier or diluent comprising a colloidal dispersion system. Fritz et al. further teach that oligonucleotides, in combination with steric stabilizers, exhibit high colloidal stability with low toxic side effects as required for biological experiments in cell culture and *in vivo* (see page 287, last paragraph).

It would have been *prima facie* obvious to one of ordinary skill in the art to target and inhibit the expression of extracellular-signal-regulated kinase-6 because the prior art has taught antisense oligonucleotides targeting extracellular-signal-regulated kinase-6 mRNA can inhibit extracellular-signal-regulated kinase-6 expression (see Dinev et al. and Mercola, D.). One of ordinary skill in the art would have been motivated to inhibit the expression of extracellular-signal-regulated kinase-6 since the prior art has taught that extracellular-signal-regulated kinase-6 is critical for myogenic differentiation (Dinev et al.) and inhibiting extracellular-signal-regulated kinase-6 can inhibit a stress activated protein pathway (Mercola, D). One of ordinary skill in the art would have expected success in making a compound 8 to 80 nucleobases in length targeted to a nucleic acid molecule encoding extracellular-signal-regulated kinase-6 since the prior art has taught extracellular-signal-regulated kinase-6 nucleic acids (Lechner et al.) and the prior art has taught antisense nucleic acids targeting extracellular-signal-regulated kinase-6 (see Mercola, D. and Dinev et al.). One of ordinary skill in the art would have been motivated to modify antisense oligonucleotides targeting extracellular-signal-regulated kinase-6 because the prior art has taught the desirability of such oligonucleotides are often preferred over native forms because of enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases and the exhibition of high colloidal stability with low toxic side effects as required for biological experiments (Baracchini et al. and Fritz et al.).

Therefore, the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.